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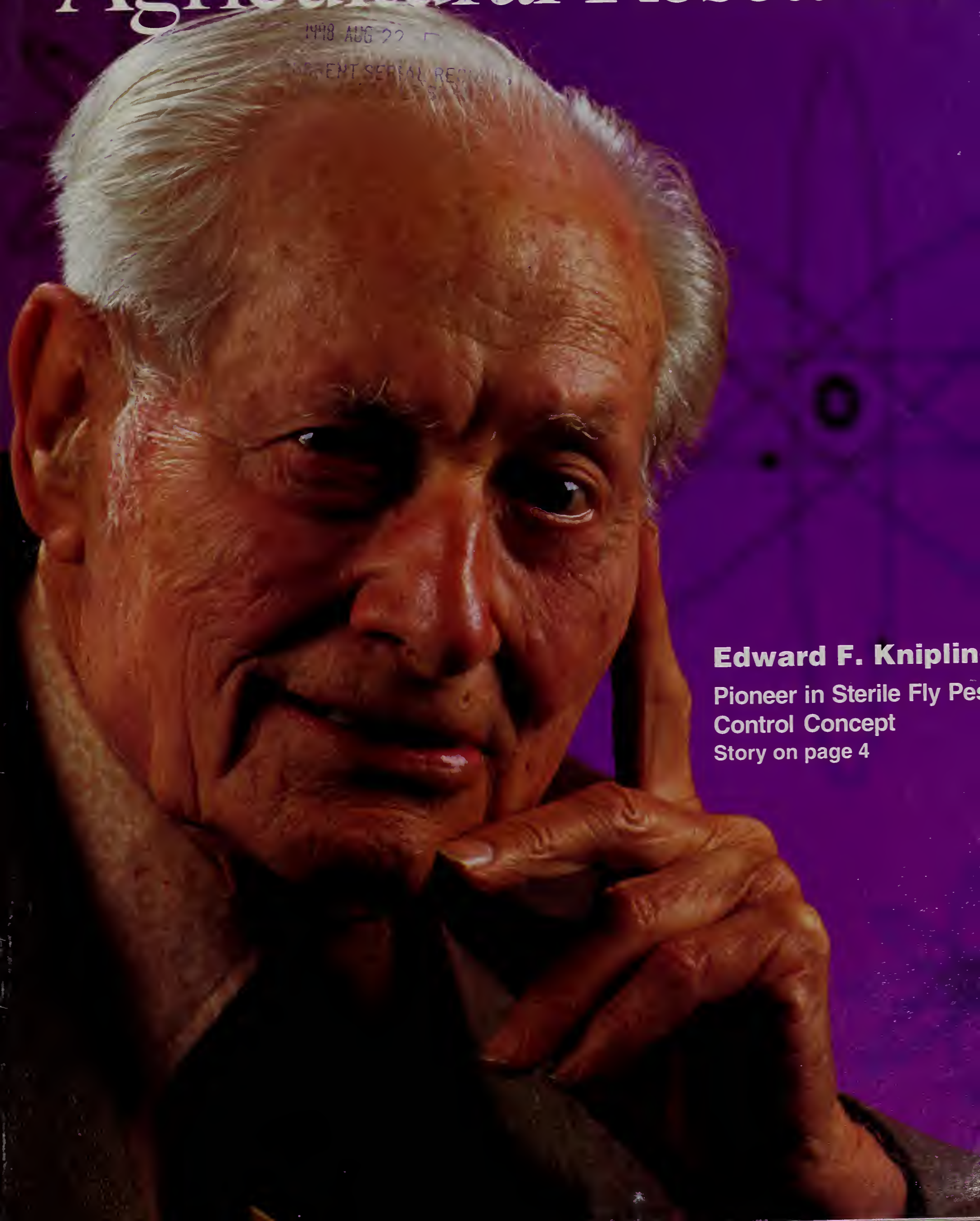
Agricultural Research Service

July 1992

# Agricultural Research

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**Edward F. Knipling**

Pioneer in Sterile Fly Pest  
Control Concept

Story on page 4



## ***The Working Month of July***

July is generally not an idyllic time for farmers. While many Americans are taking time off for vacation, farmers roll up their sleeves for some of the year's most intensive work. Crops are growing, but so are insect numbers. Turn a few leaves, and you have an instant status report on which pest-control problems need to be addressed—and addressed right now—if the coming harvest is to be a profitable one.

When insect populations burgeon, critical dollars-and-cents decisions must be made quickly. Set the farmer's day-to-day and season-to-season production deadlines against agriculture's commitment to long-term environmental risk reduction, and you can appreciate why many producers feel they have their hands full right now. And why they look to agricultural research for viable pest control plans that serve their immediate needs while remaining ecologically benign.

July's feature story, "20th Century Insect Control" by Sandy Miller Hays, chronicles the course of modern thinking in insect control, from 19th century successes in biocontrol to the discovery of DDT, which proved a lifesaver during World War II when an epidemic of typhus loomed over Europe. The article walks us through dark days of disillusionment with the ecological risks posed by hydrocarbon chemicals and examines the thinking behind today's more ecologically sensitive insect control strategies that focus on total population management.

\* \* \*

A few years back, orchardists whose trees were plagued by fire blight received some heartening news. ARS scientists had discovered an environmentally friendly bacterium that could outcompete the troublesome disease pathogen. But discovering this helpful bioagent proved only a first step toward developing a usable control for fire blight. Scientists still needed a way to safely and effectively broadcast the good bacterium to every nook and cranny of the orchard.

Just then, an old familiar friend happened to buzz by...*Apis mellifera*, the humble, hardworking honey bee. At that moment, researchers at the ARS Bee Biology and Systematics Laboratory in Logan, Utah, had a honey of an idea.

Well, it didn't happen quite that way. What it took was a specially rigged exit lane from the beehive, one in which each honey bee is automatically dusted with the biocontrol bacteria—and *voila!* At every stopping point throughout the orchard, a pollinated blossom becomes an inoculated blossom, and a colony of fire-blight-fighter bacteria is on its way to becoming established. Story by Julie Corliss and Sean Adams on page 10.

\* \* \*

When you're out in the middle of a fallow field, and the wind is swirling dirt all around you, the laws of physics may be the last thing on your mind.

But ARS scientists are taking a more calculating look at what causes dust storms to be the sudden hazards that periodically plague us, leading to crop loss, lung and eye irritations, and even tragic traffic accidents.

The scientists, at the ARS Conservation and Production Systems Research Unit in Big Spring, Texas, have unearthed some fascinating findings. It turns out that a delicate dance between wind, soil, and moisture conditions is played out in a complex cycle of events.

They've found, surprisingly, that marble-sized aggregates of earth—small clods, if you will—become agitated by the wind during an oncoming dust storm. Though they may be kicked along only a few feet, these clumps of dirt are more damaging than soil scientists had previously believed. Stephen Miller tracks the life cycle of a dust storm on page 28.

\* \* \*

Getting the most from forage is especially important for dairy cows because they have higher energy requirements than beef cows. So wouldn't it be great if Bossy could, as reporter Linda Cooke puts it in her page 20 story, "get more benefit from every bite"?

It's been long known that lignin—a cell wall component that gives plants their structural strength—resists digestion. At several USDA-ARS locations, researchers are coming up with ways to enhance both forage production and use. So, while some scientists approach forage digestibility from the viewpoint of changing the growing plant, others are devising ways to improve digestibility after the crop is harvested.

\* \* \*

You don't need to be a crop scientist to know that, on a sizzling hot afternoon in late July, certain crops appear to be suffering more than others from the heat. Check out the vigor of your corn, then look over at the tomatoes that seem to be drooping.

Just as a person who prefers the cool afternoons of upstate New York might wilt in midday temperatures that seem ideal to an Arizonan, different species of plants have clear temperature preferences—preferences that transcend the variations between growing seasons.

While it seems uncertain whether people are born with their affinities for hot or cold weather, scientists strongly suspect that plants' thermal kinetic windows are genetically controlled, which leads to some intriguing speculation. See Don Comis' "Resetting a Plant's Thermostat" on page 14.

**Regina Wiggen**

ARS Information Staff

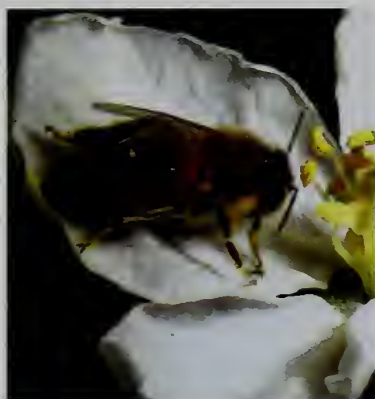


# Agricultural Research



**Cover:** Edward F. Knipling (retired) is perhaps best known for originating the concept of pest suppression by the release of sterile insects among native insect populations. The technique was first used to eradicate screwworm, the most damaging insect pest of livestock and wildlife in the United States and Mexico, and is now being used against other serious pests elsewhere in the world.

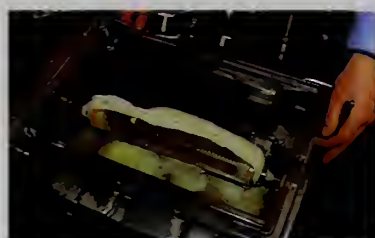
Photo by Scott Bauer (K4722-1)



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# 20th Century Insect Control

During the past 100 years, scientific approaches to insect pest control have run a gamut from the naively simple to the technologically complex. But the underlying spur to these diverse research efforts has been a single immutable fact: The successful production and storage of the world's agricultural crops—and the effective protection of humans and livestock against insect-carried diseases—depend upon the sus-

tained control of hundreds of varied and adaptable insect pests. So, even after a dynamic century of discovery and innovation, the earnest quest for safe, economical insect control methods still continues. Much has been accomplished...and yet, so much more remains to be done...if agriculturists are to have the means available to defend against the predations of these widespread and destructive pests.





**T**he storm clouds of World War II had broken wide open over Europe, and the U.S. Army needed help with a big problem that came in a tiny package.

It is a sad fact of life that where there are people crowded into unsanitary conditions, there are likely to be body lice...and in the wake of those lice often comes typhus. Thus it was for the refugees of war in the Europe of the early 1940's.

Fortunately for the suffering Europeans, U.S. Department of Agriculture scientists had begun some special research in 1942 at Orlando, Florida, at the request of the U.S. Army, which was worried about the welfare of its troops as well as civilians.

Twenty-five men at Orlando bravely allowed themselves to be infested by some of the research station's colony of 75,000 lice in tests seeking an effective control against the vermin.

Those volunteers did not squirm in vain. Within 4 months, Formula MYL was developed, and soon millions of 2-ounce cans of the powder were on their way to American and Allied troops.

The human guinea pigs at Orlando weren't the only ones contributing to the effort. Edward F. Knipling, retired director of the Entomology Division of USDA's Agricultural Research Service, recalls how chemical companies sent in hundreds of samples of their wares to be checked for possible use against not only lice, but also mosquitoes, ticks, fleas, and bedbugs.

So it was that a packet of DDT arrived at the Orlando lab in early 1943 from the Geigy Co. in Switzerland.

"We put it in a test for body lice, and its lasting properties were at least twice those of the powder we'd developed," Knipling says. "It appeared to be quite safe, and in a matter of months we recommended its use by the military. Millions of men, women, and children were soon dusted for lice in the war theaters.

"Then we tested it for every other insect we were working on—house flies, mosquitoes, bedbugs. It gave fantastic results on practically everything.

"The phenomenal results with DDT stimulated industry in the United States, Great Britain, and probably other places to look for related types of chemicals," says Knipling. "By the late 1940's, there were several other encouraging insecticides available: benzene hexachloride, chlordane,

An early hero of biological control was C.V. Riley, head of USDA's Division of Entomology upon its establishment in 1881. Prior to his USDA post—while still Missouri's state entomologist—Riley made the very first international shipment of an insect natural enemy when he sent specimens of a predaceous mite to France to control grape phylloxera there.

Riley was the guiding force behind one of the United States' earliest and most notable successes in biological



Early insecticides were often dispersed with little concern about human exposure or potential toxicity.

toxaphene, aldrin, dieldrin, to name a few of the chlorinated hydrocarbons."

The modern age of agricultural chemicals had begun...to the detriment of many years of research on other alternatives.

For even before the 1940's, humans had not been entirely helpless against insects. By the time the term "biological control" was coined in 1919, scientists had already been pursuing the practice for several years.

Researchers still argue about how biological control should be defined, but one simple explanation is that it is the use of parasites, predators, and pathogens to combat unwanted invaders in crops—whether those invaders are insects, weeds, or even plant diseases.

control: importation in 1888-89 of the Vedalia beetle, *Rodolia cardinalis*, from Australia into California to battle the cottony-cushion scale, a pest threatening California's then-fledgling citrus industry.

The road to biological control looked wide and smooth in those early years. USDA explorations in Europe and Japan beginning in 1905 turned up natural enemies of the gypsy moth and brown-tail moth.

From 1905 to 1918, USDA's Bureau of Entomology imported parasites and predators of the elm leaf beetle, alfalfa and clover leaf weevils, and sugarcane borer. In 1919, the department established a laboratory at Auch, France, to simplify the search for



natural weapons; a second laboratory was established in Japan in 1922.

Biocontrol scored big again when a USDA scientist, Samson R. Dutky, developed a way to produce spores of *Bacillus popilliae*, bacterial milky spore disease, to provide effective suppression of the Japanese beetle—the first commercial microbial pesticide.

But gradually, interest waned. Research papers published on biological control equalled in number papers on insecticide research in 1915; by 1925, papers on insecticides outnumbered those on biocontrol three to one.

The events of World War II chilled biocontrol progress even further, according to Clarence H. Hoffman,

who worked with Knipling and later served as director of ARS' Entomology Division.

"There was always recognition of biological control, but I think it wasn't widely pursued as much as insecticides because when pesticides came along, they were so potent and so cheap," says Hoffman, now retired.

"When DDT came in, everything exploded. Found to be effective against over 500 pests, it was recognized as a real miracle."

Says Hoffman, "DDT set off a whole series of investigations to get related compounds. The chemists had a field day."

"Chemical companies began putting a lot of emphasis on developing

insecticides for agriculture and other purposes," adds Knipling.

"They came up with a class of insecticides called organophosphate compounds—malathion was one, and methyl parathion, and several others—that, from the standpoint of insect control, were even more effective than the chlorinated hydrocarbons that had looked so good."

In the post-World War II years and into the era of the Korean War, ARS continued cooperating with the chemical industry in evaluating pesticides, Knipling recalls.

"Most of the companies by then had their own screening programs and didn't just send us everything on their shelves," he says. "They'd only

## Subduing the Screwworm

It's one of the greatest entomological success stories of all times and also one of the least well-known peaceful uses of atomic energy: perfection of an effective control for screwworm, *Cochliomyia hominivorax* (Coquerel), using x-radiation.

Obnoxious and destructive, the screwworm is the only insect known to consume the living flesh of warm-blooded animals. It has caused immeasurable suffering and losses in livestock, wildlife, and even human populations the world over.

In the 1930's, ARS scientists Edward F. Knipling and Raymond C. Bushland turned their attention to alternative screwworm control measures. They concluded that reducing or eliminating the insect population would be a better solution than treating the pest topically after entry into hosts via skin wounds, as was then commonly done. Though World War II's pressing entomological research needs intervened, Knipling never gave up thinking about using genetic means to control screwworms.

JOHN KUCHARSKI



Tusklike mandibles protruding from the screwworm larva's mouth rasp the flesh of living warm-blooded animals. A wound may contain hundreds of such larvae.

In 1946, he was named chief of the Insects Affecting Man and Animals Division of USDA's Bureau of Entomology and Plant Quarantine. Four years later, colleague Arthur W. Lindquist recommended to Knipling a book by Nobel laureate H.J. Muller titled "Drosophila," which discussed use of radiation to alter the genetic material of insects. Knipling immediately began a correspondence with Muller exploring the possible use of radiation to sterilize screwworms.

Convinced that the approach could work, Knipling reordered priorities to provide funding for Bushland to carry out tests at Kerrville, Texas.

Bushland secured the cooperation of a nearby U.S. Army medical unit with suitable x-ray equipment. In just 6 months, it was handily demonstrated that 2,500 to 5,000 roentgens of x-rays would sterilize screwworm pupae without disrupting their adult mating behavior.



send us the promising ones to evaluate. But we did not do a whole lot of trying to develop new chemicals; industry did that."

Besides, Knipling adds, there was already trouble on the horizon.

"By the early 1950's, it was becoming quite clear that, useful as these insecticides were, there were a lot of problems with their extended use," he notes.

"Wildlife biologists were becoming concerned with effects on wildlife. Chemicals were upsetting nature's balance, killing the natural biocontrol agents along with the pests.

"People were finding residues in meat and milk by the early 1950's, and while we were controlling some types

CHUCK HERRON/APHIS



Insecticide in 55-gallon drums readied for aerial application against a 1960's outbreak of grasshoppers in the southwestern United States.

Knipling's theory was simple: Fertile females would mate with sterilized males mass-reared in insectaries and released into infested areas. With offspring resulting only from matings with native, unsterilized males, the screwworm population would gradually become insignificant and perhaps disappear.

But could it really work? First, a field test on Sanibel, the 20-square-mile Florida island, confirmed the theory. Though encouraged, scientists knew that a larger test was needed to verify those early findings. By chance, a routine request from a veterinarian from Curacao, Netherlands Antilles, alerted them to the screwworm's presence on the 170-square-mile island. The Dutch government was eager to assist in its elimination.

So a thousand sterile flies per square mile were released each week by airplane. After just 3 weeks—the length of one screwworm reproductive cycle—about 70 per cent of new

egg cases found were sterile. After the next 3 weeks of releases, sterility was 84 percent. And by the end of the third 3-week period, very few egg masses were to be found and all were sterile!

The speed with which screwworm eradication was achieved on Curacao demonstrated the great potential of this control method. Since then, strategic deployment of sterile flies has been used effectively in many locales—most recently, northern Africa—to protect vast areas from the horrific screwworm's predations.

Ever the visionary, Knipling believes that the sterile fly technique can be a successful management tool for many other insects of economic importance. "It's just a matter of working out a few more details," he says. He and Bushland were recently honored for their screwworm research by the United Nations' Food and Agriculture Organization in Rome, Italy.—By **L.R.**

**McElreath, ARS.**

### Shedding Light on Parasite/Host Relations

Just off press is E.F. Knipling's latest contribution to insect pest control, "Principles of Insect Parasitism Analyzed From New Perspectives." Subtitled "Practical Implications for Regulating Insect Populations by Biological Means," the 338-page book sheds light on ill-understood parasitoid/host interactions. Application of its principles could move the world closer to more effective, economical, and environmentally safe pest management.

The book is available for \$9.50, including postage and handling, from the Superintendent of Documents, P.O. Box 371954, Pittsburgh, PA 15250-7954. Make checks payable to Superintendent of Documents and specify both stock number S/N 001-000-04582-2 and order processing code number 6237.





Geneticist Phyllis Martin sprays a new Bt solution on tomato plants to determine its toxicity to the Colorado potato beetle. Since Bt is harmless to humans, its use does not necessitate protective clothing. (K2785-2)

of insects, the overall insect problem continued to be about the same, year after year. So there were good indications that insecticides alone wouldn't be the long-range solution."

Jack R. Coulson, now an entomologist in ARS' Insect Biocontrol Laboratory at Beltsville, Maryland, was still a college student in 1949-50, when he landed an unusual summer job: feeding DDT to quail and pheasants in experiments at the U.S. Fish and Wildlife Service's Patuxent Wildlife Research Center at Laurel, Maryland.

"Even then, scientists were concerned about its getting into the food of game birds and were studying the effects of that," Coulson recalls. "Scientists were also aware of what was happening with development of pesticide resistance among insects."

And even earlier, in 1947, Clarence Hoffman had worked on a research team in Pennsylvania testing the widespread effects of aerial applications of DDT against forest insects.

"We were looking at how much you could use and be safe, using from 1 to

5 pounds per acre," Hoffman says. "I tell you, those 5 pounds per acre were really destructive. We entomologists were the first to find that birds were very vulnerable to DDT."

Among those in the scientific community who saw the handwriting on the wall was ARS' Knippling. He recalls that he began making shifts in ARS' entomology research program to do more on biocontrol, host plant resistance, changes in cultural practices, and pest attractants.

"In 5 years, we'd shifted from 80 percent of our effort being on insecticides to probably not more than 50 percent," he says. "We were recommending to USDA and others that there should be more effort on biological control and other alternative methods, but our appropriations were not increased."

Richard S. Soper, now the ARS national program leader for biocontrol, recalls Knippling's spreading the word in the 1960's.

"I can remember when I was a graduate student at Cornell and he came

by and spoke to us," says Soper. "Dr. Knippling changed policy, going from the treadmill-like screening of insecticides and pesticides to looking at a different approach."

In 1962, Knippling's campaign got a healthy boost from the publication of the book "Silent Spring," written by Rachel Carson, that sounded a widespread warning about the environmental drawbacks of chemical use.

"'Silent Spring' got me involved in biocontrol," notes Soper. "I was writing a graduate thesis on wood-boring beetles, and the best control I found was a fungal pathogen. When 'Silent Spring' came out, everyone was concerned."

"Her book probably did more than anything else to gain public attention and convince the budget people that these pesticides had caused problems and we needed to look more at biological controls," adds Knippling.

But if those who held the purse strings needed convincing, many scientists did not. "Ed Knippling was already well on

FRED WITTE



A Mexican bean beetle larva becomes a meal for the spined soldier bug. (K1461-3)



his way to developing nonchemical control methods when 'Silent Spring' came out," Hoffman says.

USDA research on biological control and other alternatives to chemical insecticides boomed in the 1960's. New labs sprang up in Columbia, Missouri; Gainesville, Florida; and Fargo, North Dakota. Plant breeders concentrated more effort on developing plants with the natural ability to resist or tolerate pest attacks.

"It was almost a complete shift—80 percent of our effort was on alternatives to chemicals to control insects," says Knipling.

The successes were impressive, and led to significant savings for farmers. In 1986, it was calculated that biocontrols against the alfalfa weevil netted savings of about \$48 million annually; a later report put the benefit even higher, at \$88 million in 1987 dollars. And the price tag for the research was a mere \$1 million—for a ratio of return on investment of about 50 to 1. Savings from biocontrol of another pest, the pea aphid, were calculated in 1984 at about \$36 million annually—on alfalfa alone.

In a book he is currently editing on the first century of USDA's biological control research, Coulson writes that since 1953, USDA's classical biocontrol program has saved growers several hundreds of millions of dollars annually, largely as a result of reduced cost of pesticide applications. This would put total grower savings from biocontrol during the past two decades at about \$2 billion or more.

It's not surprising that, today, thoughts often tend to turn to biological controls as weapons against the relentless invasion of pests. But biological controls, for all their virtues, are no panacea, warns Coulson.

"We get a new major pest in this country about every 3 years," he says.

"Biocontrol alone will have a hard time keeping up."

In fact, one of the chief criticisms of biological control is that it moves too slowly—and does too little. Even its strongest supporters acknowledge that natural biological agents will not adequately control every insect and that they take time to work.

"Our goal isn't 100-percent control—or at least, it shouldn't be," notes Soper. "The goal is to produce a crop economically. You only have to bring the pest population levels below the economic threshold—that point at which the farmer starts to lose money because of the pest."

Farmers in the future should go one step further, says Knipling, to a concept

he calls "total population management." This entails widespread, coordinated attacks on populations of the major pests at the precise time when such attacks will damage them most.

But even that won't allow a total farewell to chemicals, Knipling warns.

"We'll always need insecticides," he says. "In the United States alone, there are probably 1,000 different insects a year that cause a little bit of damage somewhere."

"But probably 100 of those insects cause damage year after year across a much wider area. It's those we should plan to control in an ecologically sound manner before they become an even bigger problem."—By **Sandy Miller Hays**, ARS.

TIM MCCABE



Before being released in the United States, all candidate biocontrol insects—such as the flea beetles under observation here by entomologist Gaetano Campobasso—are caged with as many as 65 different crop and ornamental plants to make sure they do not attack nontarget species. (2933-14)



# A Beeline to Biocontrol

A honey bee delivers fire-blight-fighting bacteria to an apple blossom. (K4716-1)

**A**s if the honey bee didn't have enough to do already.

Perhaps nature's hardest working insects, bees are usually busy making honey and pollinating billions of dollars worth of plants each year as they go from flower to flower in search of nectar.

Now Agricultural Research Service scientists are taking advantage of the honey bee's work habits, using it as the ultimate biological control courier. While picking up nectar and pollen, the bees (*Apis mellifera*) are dropping off biological control organisms along the way. The targets: fire blight, a bacterial disease of pears and apples, and corn earworms, one of the worst pests of corn and cotton.

In these studies, unsuspecting bees are delivering biocontrol agents right where they're needed on the plant. If future experiments succeed, some growers might one day rely on bees to help them protect as well as pollinate their crops.

## Bees Fight the Blight

Fire blight is caused by *Erwinia amylovora*, a bacterium that first colonizes a flower's stigma—the part that receives pollen grains. Although disease outbreaks are quite erratic, warm, wet weather provides prime conditions for *E. amylovora* to grow. The pathogen can spread quickly, forming cankers on twigs and branches, leaving trees with a burnt appearance; hence, the name fire blight. In some cases, infested trees may die.

At the ARS Bee Biology and Systematics Laboratory in Logan, Utah, bee expert John D. Vandenberg is collaborating with Sherman V. Thomson, a plant pathologist working on fire blight biocontrol at Utah State University.

In the 1980's, scientists discovered that spraying trees with high populations of beneficial bacteria could help



prevent the disease. These bacteria outcompete *E. amylovora* bacteria on the nutrient-rich, moist stigma, so the pathogen can't gain a foothold and invade the tree.

The idea for enlisting bees to spread fire blight-fighting bacteria goes back to a century-old finding, says Thomson. In the USDA's 1895 Yearbook of Agriculture, Merton B. Waite published a study showing that bees—along with ants, beetles, and several other insects—disperse *E. amylovora* among flower blossoms.

"I thought, if bees can disseminate the pathogen, why not use them to spread the biocontrol bacteria?" says Thomson.

Since orchardists use bees for pollinating, some of the experimental design was already in place. Each spring, many growers rent hives from beekeepers. These hives are large wooden boxes containing bee colonies, abuzz with anywhere from 10,000 to 100,000 bees.

"On a typical spring day when apple trees are in bloom, bees may be active from midmorning to midafternoon, when it's nice and warm outside," says Vandenberg. During that time, a bee may make several foraging trips from the hive, visiting as many as 100 blossoms each hour.

To automatically dust the bees with the biocontrol bacteria before they head for the flowers, the scientists used a pollen insert, a device already employed to enhance cross-pollination by some orchardists. Cross-pollination, the transfer of pollen from one flower to a genetically different one, fertilizes the flower so the fruit will develop.

The insert attaches to the beehive's entrance, forcing bees to exit through a flat, shallow tray as they leave the hive. Pollen spread in the bottom of the tray sticks to hairs on the bees' bodies.

"For our experiments, we mixed bacteria with either apple blossom or

JACK DYKINGA



Utah State University plant pathologist Sherman Thomson (left) and John Vandenberg examine samples of fire-blighted pear blossoms and healthy blossoms. (K4718-4)

cattail pollen," says Vandenberg. The latter, collected from nearby marshes by simply shaking cattails in a plastic bag, had the advantage of being free and abundant.

The bacteria are called *Pseudomonas fluorescens* A506 and *Erwinia herbicola* 318. Neither appears to harm the bees, says Vandenberg, noting that the commonly used bacterial pesticide *Bacillus thuringiensis* is considered harmless to bees.

Last year's experiments proved the bees to be an effective method for spreading the bacteria. "After just 48 hours," Thomson says, "we found the *E. herbicola* bacteria in 92 percent of 50 blossoms taken randomly through-

out one 5-acre apple orchard." The weather on those 2 days was excellent for bee activity, he adds.

In another test on pears, they found that 72 percent of the blossoms had sizable populations of the good bacteria, in an area 20 feet from the hive.

A cold snap that froze the blossoms that year prevented a fair trial of the fire-blight control. But this spring, Thomson and Vandenberg tested the bee method again, on a total of 25 acres of pears and apples in Utah.

"We're working with four cooperating growers in two local counties," says Thomson. "If we're fortunate, the weather conditions will be right so we can see how well this treatment actually works."





Entomologist Harry Gross places a powder containing *Heliothis* nuclear polyhedrosis virus into a tray for dissemination by the honey bees. (K4703-12)

Meanwhile, in Corvallis, Oregon State University and ARS scientists are also using bees to study fire blight. But they're dousing the bees with the fire blight bacteria itself, instead of the bacteria meant to fight it.

"We're using the bees to mimic a natural infection of fire blight," explains plant pathologist Joyce E. Loper, of the ARS Horticultural Crops Research Unit. Because fire blight infection is unpredictable, the scientists wanted to find a way to test whether or not the biocontrol tactics were working, rather than wait for a natural outbreak.

She and fellow plant pathologists Virginia O. Stockwell (ARS) and Ken Johnson (OSU) are also working with *P. fluorescens* strain A506 and a different strain of *E. herbicola*, C9-1. Their treatment plans include spray applications of both bacteria, alone and

in combination, and sometimes with different antibiotics.

Currently, growers continue to spray antibiotics to suppress the fire blight bacteria, but resistant strains of the pathogen have made that practice less effective, says Loper.

Johnson set up a huge screen cage that completely enclosed 40 pear trees. When trees first began to bloom, he placed a bee hive in the cage, complete with a pollen insert that contained not pollen, but freeze-dried (yet still infectious) fire blight bacteria mixed with powdered milk and xanthan gum.

Twice during the blooming period, researchers sprayed the trees with either a mixture of the biocontrol bacteria, an antibiotic (streptomycin), or plain water. Just before full bloom, the scientists found the fire blight bacteria in 43 percent of the water-treated blossoms, confirming that the bees were indeed carrying the pathogen to the trees.

What's more, they also showed that the biocontrol-treated blossoms were able to reduce the growth of fire blight bacteria on flowers. "With this disease," says Stockwell, "there has to be a certain amount of *E. amylovora* present—about 1 million cells per flower—to cause fire blight. We found that only about 3 percent of the blossoms sprayed with the beneficial bacteria had pathogen populations that high, compared to 20 percent of the water-treated controls."

Although the approaches of the two teams differ, they share the same goal. "The exciting thing about these studies," says Loper, "is their interdisciplinary approach—getting entomologists and pathologists working together on biological control. It's really helped advance the field." While working with biological control agents against the corn earworm in the mid-1980's, Harry R. Gross, an entomologist with ARS in Tifton, Georgia, was watching honey

bees busily foraging for nectar in a field of crimson clover.

Then it dawned on him: What better way to carry insect pathogens into a field than via a honey bee? "Here's an ideal vehicle that is going right where we want the biological control agent to go," he said. So Gross and fellow Tifton entomologists John J. Hamm and James E. Carpenter began to work on a way to enlist bees in their battle against the corn earworm. The large, green-to-brown caterpillars cause an estimated \$500 million damage each year to corn and cotton in the southeastern United States alone.

### Bees Battle the Earworm

They chose a natural virus that infects only the corn earworm and tobacco budworm, but doesn't harm honey bees. Called a *Heliothis* nuclear polyhedrosis virus, it attacks the larvae and dissolves them, turning them to liquid, Gross says.

Instead of using a pollen insert to get the virus into the field, Gross developed a special device that works slightly differently. The structure allows the bees to enter and exit the hive through separate pathways.

Bees enter the hive through the bottom of the device and pass through a wire mesh to reach the colony. As they exit, they respond to light entering the hive through clear plastic panels in the top part of the device. The panel forces the bees to move downward into a metal tray that contains the virus in a fine powder. The bees' legs and underbodies become covered with the pathogen powder as they go back to the field, carrying the virus with them.

The scientists tried the technique with two hives rented from a local beekeeper in two 1-acre crimson clover fields near Tifton.

After letting the bees forage in the clover for a day, scientists collected the





By isolating some apple trees and branches, entomologist John Vandenberg can determine the effectiveness of honey bee distribution of anti-fire-blight bacteria. (K4715-1)

clover heads and fed them to corn earworm larvae in the laboratory.

The result: Between 70 and 90 percent of the larvae died, compared to between 10 and 15 percent mortality of larvae put on clover heads that had not been visited by virus-treated bees.

Gross says the technique isn't limited to using only one virus against the corn earworm. "What you choose to put in the hive is limited only by the user's imagination and by how it'll affect the bees," he says.

The technique could likely be used against any insect that feeds on flowering plants that bees visit, Gross adds, although he's not planning to continue the studies himself. "We used clover

simply as a model to test the feasibility of the approach," he says.

However, controlling earworms in clover might indirectly benefit other crops. The caterpillar pests feed on clover and other early season plants before they move on to cotton, corn, and other crops.

Bees aren't normally used in corn or cotton fields; corn is wind-pollinated, and most cotton varieties don't require cross-pollination. But bees do like corn pollen and cotton flower nectar—so they might still work as transporters of biological control.—By **Julie Corliss** and **Sean Adams**, ARS.

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# Resetting a Plant's Thermostat

**W**e worry about a change of only 2 degrees with global warming when, every day, plants are stressed and stunted by changes of 2 to 10 or more degrees, says John J. Burke, a plant physiologist with USDA's Agricultural Research Service in Lubbock, Texas.

These daily temperature fluctuations may rob farmers of two-thirds of their possible yields, he adds.

Burke is a member of a biotechnology team that is proposing a controversial theory: that each plant species is equipped with a different thermostat that is preset at a fairly narrow temperature range for best growth. Since the thermostat is set genetically, scientists are searching for a way to reset it through gene transfer—in effect creating all-weather plants with higher yields.

Their theory was supported recently by the successful transfer of a gene from cucumbers—a warm-season crop, to tobacco—a cool-season crop.

Says Burke, "According to our tests, tobacco grows best at 66°F to 82°F. For cucumbers, it's 81°F to 101°F."

But once the cucumber gene was inserted into tobacco by colleague Melvin J. Oliver, the added genetic code enabled tobacco to produce the warm version of hydroxypyruvate reductase, an enzyme that plays a role in protecting plants from heat stress. Since the altered tobacco plants also retain their cool version of the enzyme, they have a combined temperature range of 66°F to 101°F.

The cucumber version of the enzyme was found to infiltrate every part of the tobacco offspring, from the tips of the roots to the top leaves and stalk. The tobacco enzyme was everywhere in the plant except the roots and was always found with the cucumber enzyme.

The scientists extracted the enzymes from all parts of the genetically modified plants. Then they compared their properties at various temperatures.

"During the heat of day, the enzyme mixture found in the shoots behaves as it does in cucumbers; during the night, it behaves as in tobacco," Burke says. But the root enzyme always behaves as a cucumber protein.

Unfortunately, the cucumber gene plays only a small role in determining a plant's overall temperature preference. So the altered tobacco plant does not behave like a cucumber plant under a hot sun.

But Burke says the success gives the Lubbock team the green light to search for one or more controlling genes that can reset the thermostat of an important metabolic pathway to a broader temperature range, making crop plants both cool season and warm season.

## The New Theory Evolves

"The inherited change in enzyme behavior is a key confirmation of our thermal kinetic window theory," says Burke.

He and plant physiologist James R. Mahan startled the scientific world 3 years ago with this theory, which was based on the temperature reactions of two common enzymes extracted from several crops. One enzyme was hydroxypyruvate reductase; the other, glutathione reductase, which plays a similar role in protecting plants from heat stress.

At scientific meetings, skeptical colleagues asked, "How can you judge the temperature reaction of an entire plant by just observing two of the thousands of enzymes that regulate its metabolism?"

At one of these conferences, Burke was showered with good suggestions, as well as disbelief. He returned to his lab with new ideas that helped him devise two simple tests to gauge plant metabolism at different temperatures.

One test used a fluorescence meter to monitor the amount of light emitted

by plant chlorophyll, the green pigment. Leaf disks were punched out of plants growing in the field or greenhouse and brought to the lab. The disks were placed under lights for 5 minutes and then transferred to temperature plates and kept in the dark in a temperature chamber devised by Burke. Later, the disks were analyzed with the meter to identify the temperature providing the most fluorescence when exposed to a fluorimeter beam.

A second test involved a visual check of greening in seedlings. The

JACK DYKINGA



Plant physiologist John Burke observes the effects of different temperatures on the greening of seedlings. (K4712-1)

seeds were germinated in the dark, in chambers set to the optimal temperature for growth. After several days, the top two leaves of the seedling (cotyledons) were removed and exposed to light for 24 hours on plates set to different temperatures, to identify the one that stimulated the most chlorophyll accumulation.

In both tests, the plant leaves responded to temperature changes the same way the two isolated enzymes



had responded. They both showed the same narrow temperature range for best plant growth or greening, by plant species.

"These tests allow us to screen for temperature preferences in 2-degree increments, instead of the 9-degree increments possible with the isolated enzyme testing," Burke says. Whereas once he computed the optimal growth temperature range for cucumbers at 86°F to 96°F, he is now confident that it is 86°F to 90°F.

The visual greening test—much simpler than enzyme isolation—revealed the importance of the amount of energy stored up in seeds, Burke says.

"It also showed that the amount of stored energy reserves plays a major role in determining the breadth of a seedling's temperature preference.

"Anything that increases the availability of energy reserves for plant enzymes to act on means that more seedlings may survive both the ordeal of breaking through the soil surface and subsequent temperature extremes," Burke explains. Those that break through will also turn green faster during the first critical hours and stand a better chance of yielding a crop.

"For plants, it's a race against time. The seedling must sprout underground, quickly break through the soil surface to reach sunlight, and build the machinery to turn green and start making its own food. Until a plant turns green, it's living off whatever sugars and other energy sources were stored in its seed, the amounts of which are genetically determined."

Burke conjures up the image of a pale seedling straining to push aside clods of dirt before it uses up all its energy and dies. Farmers and gardeners both experience the disappointment of scanning soil for signs of life and seeing only sparse green seedlings.

"We're explaining the reasons for such seedling failure and finding

ways to change plants so they work better," he says.

By watching seeds in action, Burke realized that by improving the availability of food within the seed, breeders could compensate for the inherited inefficiency of some plants' sprouting metabolisms.

"Breeders must be sure a plant's reserves are almost used up before determining the optimal greening temperatures, or their results will be skewed by the effect of the reserves," Burke says.

JACK DYKINGA



Plant physiologist Donn Warner compares the sizes of cotton plants exposed to different night temperatures; less growth occurred with cooler temperatures. (K4711-1)

He found that the longer that plants stayed in the dark, the narrower their temperature optimum for greening became. Cotton plants, for example, turned sufficiently green within a range of 68°F to 104°F after 5 days in the dark. That range narrowed to 77°F to 86°F after 8 days of darkness.

Through trial and error, Burke determined the right number of days to leave seedlings in the dark to use

up energy reserves and eliminate their effect.

He also realized that greening tests used by breeders to select varieties that turn green the fastest fail to show the consequence of temperature on plant growth when the tests are performed just at room temperature.

### Customizing Plants for All Seasons

Eric A. Curry, a plant physiologist at the ARS Tree Fruit Research Laboratory in Wenatchee, Washington, is using

the fluorescence test to evaluate apple and peach trees with naturally broad temperature tolerances.

With orchard trees grown on grafted roots, even more crop-customizing is theoretically possible. Roots can be selected from the trees best able to grow in the soil temperature of a particular area, while the aboveground tree can be chosen from a variety best suited to the air temperature of the area.





To collect data in support of the thermal kinetic window theory, plant physiologist John Burke wires thermocouple sensors into cotton and cucumber plants. (K2984-9)

#### Thermal Kinetic Windows for Common Crops Temperature Range (°F) for Optimal Growth

|              |        |
|--------------|--------|
| Bell Peppers | 74-105 |
| Cotton       | 73-90  |
| Cucumbers    | 86-90  |
| Field corn   | 77-88  |
| Petunias     | 65-84  |
| Potatoes     | 62-73  |
| Soybeans     | 65-84  |
| Spinach      | 50-63  |
| Tomatoes     | 68-78  |
| Wheat        | 62-73  |

"Plants are grown in different parts of the country mostly for reasons of economics rather than plant metabolism," Burke says. He wants to alter metabolisms so that plants will be more physiologically suited to the areas they are grown in. Cotton, for example, is not suited to the nights on the Texas High Plains where, at 3,000 feet above sea level, it gets too cold and reduces cotton growth. Cotton stops growing at 58°F.

"There are very few nights when the mercury doesn't dip below 58," Oliver says. "Our goal is to custom-design important crops for different climates. If we could get cotton to produce at lower temperatures by transferring controlling genes from a crop that can tolerate cold nights, such as potatoes, it would boost yields greatly."

"For custom-designed crops, the distinction between warm- and cool-season would become meaningless," Burke says. "These crops should also be able to thrive across more plant hardiness zones."

The implications for gardeners as well as farmers are enormous. Can you imagine all the vegetables that go into a salad being grown in one location, from early spring through summer and on into late fall? How about a lettuce that no longer gives up and goes to seed in midsummer or perhaps a tomato that thrives in cool weather?—By **Don Comis**, ARS.

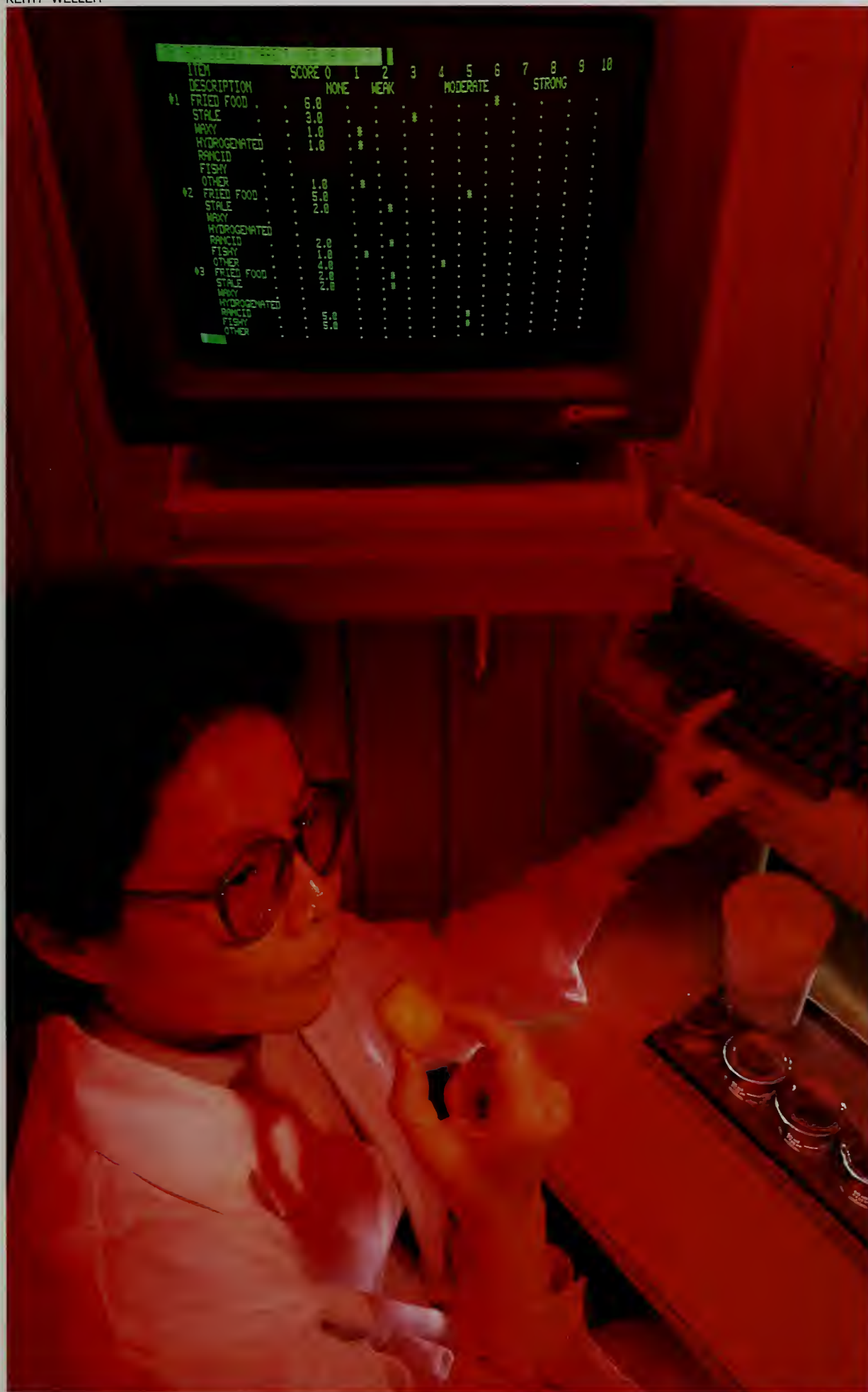
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# Soybean Oil Quality

Flavor and Aroma Testing Have Led to Its Steady Improvement

KEITH WELLER



Chemist Sharon Abidi tastes a fried bread cube and makes a computer entry of her flavor evaluation perceptions. (K4676-8)

**H**ave you ever tasted soy oil? Probably not. Oh, it's in food items like salad dressing, margarine, mayonnaise, and even Big Mac sauce. And french fries and chicken pieces are cooked in soy oil at many fast-food restaurants. Potato chips and other snacks, too, are fried in soy oil.

So why haven't you tasted it, you ask? The answer is: because it's tasteless.

But it hasn't always been this way. "Fifty years ago, soybean oil tasted like paint," recalls Herbert J. Dutton, retired ARS chemist. "It was the major problem of the soybean industry to improve the taste of soy oil."

At the request of soybean processors, ARS scientists at the National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, began to look at what caused off-flavors and to devise workable solutions. They identified trace metals from the processing equipment and naturally present linolenic acid as sources of off-flavors.

"We had been told before we started that oxidation of the oil was not responsible. But we soon found that linolenic acid did oxidize when the oil was exposed to air," recalls Dutton.

In those days, scientists didn't think that the linolenic acid content could be genetically changed through plant breeding, so they tried hydrogenation as a way to remove it from the oil. To solve the flavor problem, ARS researchers recommended two processes: hydrogenation and the addition of citric acid.

Both steps inhibited the breakdown of fatty acids when oil was exposed to air, says chemist Timothy L. Mounts, who is in charge of Food Quality and Safety Research at the center. The early researchers also recommended replacing iron processing equipment with stainless steel.

"Vegetable oils have become popular today because consumers are more health-conscious in the 1990's. People want to lower the amount of cholesterol in their food and this is a good way to



do it, since plants don't contain cholesterol," says ARS food technologist Kathleen Warner.

Warner coordinates the sensory evaluation panels at the NCAUR. Out of the 50 members on the panels, 15 are experts in sniffing the aroma of cooking oils, intensively trained in the art of recognizing characteristic odors. Some testers have as many as 20 years of experience in evaluating oils.

"Consumers don't know it, but ARS employees have done behind-the-scenes sensory evaluations since 1940 to evaluate vegetable oils and their end-use quality," says Warner.

### Testing the Taste of Vegetable Oils

In the 1940's, Dutton and others at the Peoria center came up with standardized taste tests for oils that were eventually accepted worldwide. Says Dutton, "Before then, taste-testing was a rather biased affair."

Today, the big problem is odors—odors given off during high-temperature frying with several types of vegetable oils. Odor problems stem from volatile fatty acids that break down under high temperatures and longer cooking times.

During heating, various types of oil may emit as many as 40 different characteristic odors. For example, soy and canola oils smell fishy. Corn oil has burnt or corny odors. In contrast, cottonseed oil develops a more fried-food-like aroma, so it's often used in blends with other vegetable oils to mask objectionable odors.

In the 1960's, the Peoria center began studying and evaluating soy oil. Special rooms were set aside to check aromas given off by frying oils.

At first, these rooms were just large, empty labs in which pans of heated oil were set. All exterior doors were taped shut to keep other odors out—except for one door for panel members to enter, sniff, and record their perceptions.

Later, small 5' by 8' by 10' rooms were constructed with airflow and

KEITH WELLER



Janet Snyder, a chemist and trained aroma panelist, evaluates heated oil odor which is fed into the room's odor chamber through a ventilation system. (K4675-16)



temperature controls. Panelists enter these through a 2' by 3' by 10' anteroom that serves as an airlock. Odors to be sensed are ducted in through a ceiling vent and exhausted through vents in the four bottom corners of the room. The location of the exhaust vents ensures the even mixing of the odors with the air in the room.

Several major food companies have used the Peoria aroma lab as a model for their own similar facilities.

Now, 40 years after scientists abandoned the idea of genetically changing the composition of oils, plant breeders have found they can genetically alter the seed to produce oils with modified fatty acids.

The panels' reactions to the performance of these modified oils during cooking and frying will tell plant breeders how well the modifications affect eating quality. Someday, these new-generation oils may be stable enough for frying without prior hydrogenation, says Warner.

The panel is currently evaluating oils from experimental soybean lines bred for low-linolenic acid content developed by plant breeders at Purdue, North Carolina State, and Iowa State.

In another project, panelists are evaluating soybean and canola lines developed from special breeding programs by InterMountain Canola Co. in Cinnauminson, New Jersey. A cooperative agreement between the two organizations makes this work possible.

Yes, soy oil has come a long way in the last 50 years...from a product that once tasted like paint to one with 75 percent of the U.S. market for edible oil.—By **Linda Cooke, ARS.**

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KEITH WELLER



Using nuclear magnetic resonance, research leader Timothy Mounts collects data on heated fat samples to correlate with sensory evaluation results. (K4675-8)

KEITH WELLER



Food science technician Linda Parrott prepares a sample tray of heated oils for presentation to taste panelists. (K4677-13)



# Getting the Most From Forage

It'll Cut Costs and Enhance Production

SCOTT BAUER

It may come as a surprise to many consumers that one of the trickiest ingredients of their ice cream sundae is forage.

Forages—both grasses and legumes—and grains make up the bulk of the dairy cow's daily bill of fare. But how much forage a cow eats may not be as important as how digestible that forage is. If the cow's digestive system can't break the forage down into usable nutrients, eating becomes just so much jaw exercise.

Getting the most from forage is especially important for dairy cows because they have higher energy requirements than beef cows. Milk production requires extensive energy, protein, minerals, and vitamins that are three to four times the minimum needed to just maintain a cow's body.

And getting the most from forage requires maximizing its digestibility. This allows farmers to lower production costs by either feeding less feed or changing the ration to include more high-quality forage.

The ultimate objective is not to feed Bossy more—because that would just raise input costs—but rather, to increase the digestibility of feed so that she gets more benefit from every bite.

One major stumbling block to more efficient digestion has been lignin, the tough material that permeates plant cell walls and cements the cells together.

"Lignin plays an important role in the plant's defense mechanism, helping it to withstand stress, insects, and disease," says plant physiologist Dwayne R. Buxton in Ames, Iowa.

"Without lignin, plants would lose their structure and fall over. But plant breeders would like to modify lignin's chemical makeup to increase the digestibility of that fiber," says Buxton.

The chemical make-up of lignin is different in various parts of the plant, such as in stems and leaves. Slight modifications bred into the plant could ensure enough lignin to keep the plant



Plant physiologists Dwayne Buxton and Teresa Morrison examine young corn plants being grown for research on digestibility-limiting factors. (K4599-11)



upright but make it easier for ruminant animals to digest it, says Buxton.

Buxton is part of a group of ARS researchers focused on understanding the structure and function of plant cell walls. Besides Buxton, others in the group include Hans Jung in St. Paul, Minnesota, and Ron Hatfield, Dave Mertens, Paul Weimer, and John Ralph in Madison, Wisconsin.

The plant and its nutritive value can be compared to those hollow, waxy confections that hold sweet syrup inside. Like the syrup, many of the nutrients are held inside cell walls that can be likened to the inedible waxy containers. However, the cell walls themselves also contain useful nutrients—if they can be digested.

As soon as the walls are ruptured by chewing, the digestive process begins. A thinner cell wall would digest faster and allow the cows to get to the “good stuff” inside the cell faster, says Buxton.

“Too, some types of lignin may inhibit digestion more than others. If we can figure out what causes lignin to be formed—what chemicals prompt its production—we might be able to control not only how much, but also what type of lignin is formed,” he says.

Among forages, one of the choices with the fewest drawbacks is alfalfa. Called the Queen of Forages, this legume is related to the peas and beans that humans eat. Its popularity is evidenced by the more than 26 million acres planted to this crop in the United States.

“Alfalfa is nearly the perfect feed for dairy cows,” says Buxton. “It yields well and produces more protein per acre than any other grain or oilseed crop. That makes it a highly nutritious animal feed.”

Still, alfalfa could be improved. One problem with it is that its quality declines as the plant matures, so farmers have to cut alfalfa early and harvest several times during the growing season.

“The more times farmers run their harvesting machines through the field,

the higher their cost of production,” says Buxton.

There are other reasons for making forages more nutritious. In the Midwest, planting forage grasses and legumes is increasingly important because of environmental and conservation concerns. In soil conservation programs, forages are preferred over grain crops because they are sod-forming and require fewer chemicals and less tillage. Legumes are also important because they transform atmospheric nitrogen into a form that plants can use for growth.

### Enhancing Forage Production and Use

At the U.S. Dairy Forage Research Center, which opened in January 1981 in Madison, Wisconsin, scientists have been developing ways to increase efficiency of forage production and feeding. The work of these scientists is augmented by five ARS scientists based at state agricultural

experiment stations in Iowa, Michigan, Minnesota, and New York.

Forage production in the United States has an estimated value of \$10 billion annually. An improvement of only 10 percent in just one type of forage—alfalfa—could boost the overall value of forage to farmers immensely.

While some scientists approach forage digestibility from the viewpoint of changing the growing plant, agricultural engineer Richard G. Koegel has devised a way to improve digestibility after the crop is harvested.

Koegel and others at Madison designed a machine to more efficiently shred alfalfa hay for fast drying. This also makes the hay easier for cows to digest.

Currently, alfalfa is either mowed, dried, and baled to make hay, or it is mowed, partially dried, and chopped to produce silage. But time lost waiting for hay to dry increases susceptibility to weather damage, which contributes to overall harvesting

BRUCE FRITZ



Using a scanning electron microscope, biological assistant Christine Odt examines the attachment of fiber-digesting bacteria to forage particles. Understanding how this occurs may provide clues for improving the process. (K4597-1)



losses of 20 to 30 percent of usable nutrients, Koegel says.

He says the forage mat machine is a good alternative to conventional mower-conditioners. The mat maker has rollers that crush plants and split stems—the less digestible part of the plant—into ribbons. It mashes leaves and upper stems, and a rotor further fiberizes the plants. [See *Agricultural Research*, August 1988, p. 7]

Assisting in development of the machine were ARS agricultural engineer Timothy J. Krause and University of Wisconsin agricultural engineers Richard J. Straub and Kevin J. Shinnars.

### Shredded Alfalfa Speeds Digestion

Koegel says that feed only stays in a cow's digestive tract from 24 to 48 hours, so increasing the rate of digestion enhances nutrient absorption.

By studying rumen fermentation, Koegel and ARS dairy scientist David Mertens determined how much of the feed goes toward body maintenance versus milk production.

For example, a 1,500-pound cow producing 5 gallons (43 pounds) of milk a day needs 39 pounds of feed containing 61 percent total digestible nutrients (TDN). Of this, she uses 10.3 pounds of nutrients to maintain her body and 13.4 pounds to produce her milk.

However, if a similar cow produces 15 gallons (129 pounds) of milk daily, she requires 67 pounds of feed containing 73 percent TDN. While her body maintenance needs remain the same as those of the less productive cow, she requires nearly three times the TDN to produce the extra milk. This higher TDN requirement dictates a ration containing half forage (56 percent TDN) and half grain (85 percent TDN). Just a four-percent increase in forage TDN—from 56 percent to 60 percent—would allow either a change in ration to 60 percent forage/40



BRUCE FRITZ

Dairy scientist David Mertens oversees the feeding of dairy cows to determine the optimal mixture of grain and forage and to evaluate the effect of shredding on alfalfa's feed value. (K4597-4)

percent grain, or a 2-pound reduction in intake of the original ration.

In 1990, Koegel and Mertens fed both shredded and conventional alfalfa silage to 12 dairy cows. The cows eating the silage made from shredded alfalfa gained more weight while producing the same amount of milk as those eating conventional silage.

In a later study, they placed four cows on rotational diets of alfalfa hay,

macerated alfalfa hay, alfalfa silage, and macerated alfalfa silage. Each diet was fed for 2 weeks, and findings confirmed the earlier results. By measuring the rumen fluid for volatile fatty acids, the researchers can determine how energy from feed is going to be used—either for gain in body weight or milk production, says Mertens.

Boosting the nutritive value of forages can serve two purposes. It can help dairy farmers reduce the cost of feeding without sacrificing production, and it can save natural resources by growing grasses that require fewer chemicals and don't erode the soil.—  
By **Linda Cooke**, ARS.

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BRUCE FRITZ



Agricultural engineer Richard Koegel evaluates the forage mat machine and its shredded product. (K4599-9)



# Finding the Right Rangeland Grass

JERRY COX



In Sonora, Mexico, agricultural technicians collect samples of buffelgrass.

**S**omeday, it may be possible to use a computer to select the best plant for any climate in any country, says ARS scientist Jerry R. Cox. "This could be a lifesaver for regions that barely have enough food for their populations today."

The computer program Cox talks about has yet to be developed, but he and scientists at Woodward, Oklahoma, are laying the groundwork. Cox obtained worldwide soil and climate data from the United Nations Educational, Scientific, and Cultural Organization (UNESCO) and is now using the information to locate areas that will support four particularly hardy grasses from Africa.

By using programs similar to the one that Cox is developing, people would enter soil and weather data from the

geographic region they wish to plant. The computer would then match those conditions with information on plants that thrive under those conditions.

Candidate species could be further screened with the range plant profiles (RAPPS) computer model that is currently being developed by ARS researchers at Woodward.

The model uses weather and plant physiology data to simulate range grass growth. After comparing production and long-term survival, the scientists could recommend grasses with the highest probability of success.

As a range scientist, Cox has seen firsthand the need for better assurance that range seeding will succeed. He says the world's largest range experiment started around the beginning of this century because leaders in both the

Northern and Southern Hemispheres were concerned about declining grassland productivity and increasing soil erosion.

Government employees and private individuals from Australia, England, South Africa, and the United States were enlisted in collecting and exchanging grass seed from every continent. The goal was to discover a "miracle grass" that would produce abundant forage with minimal water.

Hundreds of people planted thousands of seeds on their experiment stations and ranches. They noticed that four warm-season grasses native to Africa were easier to establish and persisted longer than grasses from other parts of the world. These four grasses, buffelgrass (*Cenchrus ciliaris*), weeping lovegrass (*Eragrostis curvula*),



## Four Exceptional Grasses

To survive and thrive, their requirements are as follows:

**Buffelgrass:** 6-20 inches summer rain, mean low winter temperature usually above 41°F, and loamy soil.

**Weeping lovegrass:** 16-40 inches of spring, summer, and fall rain; average winter temperature usually above 23°F; deep sandy soils.

**Kleingrass:** 16-39 inches of summer rain, average maximum daily summer temperature above 86°F, average winter temperature usually above 32°F, and clay or silty soils. Weeping lovegrass and kleingrass spread to nonplanted sites only in Africa, where midsummer drought does not occur.

**Lehmann lovegrass:** summer rainfall exceeding 6 inches in 30 to 40 days and sandy or sandy loam soils. Predominates and spreads only in southern Africa, southeastern Arizona, and at few sites in northern Mexico.

kleingrass (*Panicum coloratum*), and Lehmann lovegrass (*Eragrostis lehmanniana*), have dramatically increased productivity—at least, for a time—on millions of acres of depleted rangeland worldwide.

“For more than 100 years, there were thought to be only four steps to establishing perennial grasses on rangelands: clear shrubs from the land, prepare a seedbed, plant many varieties of seed, and pray for rain,” says Cox at ARS’ Forage and Range Research Unit in Logan, Utah.

But this simple set of rules produced a stand of grass in only 1 out of 10 attempts. Poor soils and scarce precipitation are formidable adversaries for tender grass seedlings.

Still, land managers kept trying in this crude way, because improved ranges were necessary to boost their profits. But in the 1970’s, private land owners facing dramatically increased fuel prices could no longer afford to reseed 10 times in order to get one successful stand.

Cox says that between 1930 and 1986, scientists in many countries

studied the four promising African grasses, but they did not attempt to create a database that would help explain why some grasses survived while others died.

“Because successes occurred infrequently, scientists and land managers tended to extrapolate prematurely whenever a species was successfully established. But often those preliminary successes could not be duplicated throughout a geographic region,” says Cox.

For example, Lehmann lovegrass can be established on most soils in the southwestern United States and northern Mexico during a wet summer. However, the grass dies out during the next few years unless soils are either sandy or sandy loam and there are 6 to 9 inches of summer rain in 30 to 40 days,” says Cox.

The initial establishment of plants from seeds was documented in 31 different countries for buffelgrass, 15 for weeping lovegrass, 9 for kleingrass, and 5 for Lehmann lovegrass. Today, buffelgrass survives only in six countries, weeping lovegrass in three,

JERRY COX



Weeping lovegrass in Texas. Today, weeping lovegrass survives in only three countries worldwide.



JERRY COX



Buffelgrass in the Arcadia Valley in northeast Australia.

kleingrass in two, and Lehmann lovegrass in one.

Because each grass is self-perpetuating on two or more continents, Cox believes that some common factors directly affect plant establishment.

"If we can match soil and climate conditions where grasses evolved to potential new seeding sites with similar conditions, then range seeding in both the Northern and Southern Hemispheres may be more successful," says Cox.

To prove his theory, Cox collected climatic information and soil samples at more than 350 sites on five continents in 1986 and 1989. Countries he visited included: Argentina, Australia, Botswana, Brazil, Kenya, Mexico, Namibia, Pakistan, South Africa, and Tanzania.

Depending on various soil and climate factors, grasses either die, grow without spreading, or grow and expand onto an ever-increasing area.

Sometimes grasses that merely thrive are better suited to a site than those that spread out. For example, if

land managers grow highly nutritious native grasses next to an area that needs reseeding, they will reseed with a grass that doesn't spread. Elsewhere, the area to be reseeded might be so vast that a spreading variety would be desirable to spare managers from having to seed every acre.

Cox recently introduced into southern Africa some 30 types of buffelgrass collected from around the world. "Our goal is to determine which ecotypes are best adapted to four climatic regions and then use genetic evaluation to determine differences in plant survival," Cox says. "If we can identify drought-tolerant genes and incorporate them into annual crops, we might be able to grow other grasses—including wheat and corn—in areas of the world where today only range grasses survive."—By **Dennis Senft, ARS.**

*Jerry R. Cox is at the USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, UT 84322-6300. Phone (801) 750-3066. ♦*

## Our Edible Staple Grasses

Of all plants, grasses are the most dietarily important to humans. Corn, wheat, oats, rye, barley, rice, and sugarcane are all grasses, the same as the plants that cover our front yards and vast rangelands.

Grasses have a special place in U.S. history. The early settlers found the land on the Great Plains—a vast area that extends from southern Canada, across the United States, to northern Mexico—practically treeless, an indication to them that the soil was somehow less valuable.

But they were wrong. Grasses can withstand environmental extremes that kill trees. So the grasses these pioneers saw were uniquely adapted to the multitude of soil, temperature, and precipitation variations and combinations present on the Plains.

On the eastern prairies, grasses were tall, lush, and deep rooted. To the west, on the High Plains, they were shorter and the soil surface was sodded. Farther west, between the Rocky Mountains and the Sierra Nevadas, the grasses grew in bunches or tufts because precipitation was limited and couldn't support extensive stands.

Vegetation east of the Rockies supported mighty herds of bison. But the building and completion of the Union Pacific Railroad in the mid-19th century opened the Plains to hunters who fed an expanding eastern population. In less than two decades, the bison herds were all but destroyed, with fewer than 1,000 remaining from an estimated 30 million.

Cattlemen soon arrived to take advantage of what seemed an unlimited grass supply. By 1880, cattle ranching was booming, financed by foreign capital that was largely European. Soon, the ranges were fully stocked and the "unlimited" grass supply was quickly dissipated.—By **Dennis Senft, ARS.**



# Filamentous Fungal Fermentations

**S**uppose a microbiologist finds a compound produced by a fungus that—if developed into a drug—could revolutionize medical treatment of a disease.

But what if the microbiologist then learns that this newly discovered compound can't be economically mass-produced using today's fermentation methods?

There are two losers in this hypothetical scenario: the company that could manufacture the pharmaceutical, and—more importantly—the user who would be denied this new treatment.

Presently, some fungi produce antibiotics such as penicillin, enzymes that convert corn syrup to sugar, and chemicals such as citric acid and industrial alcohol.

But bridging the gap between biological breakthrough and practical application may be eased by an invention developed and now being patented by ARS scientists.

Called an attached-growth biological reactor, the invention compensates for the problems certain fungi have trying to grow in standard fermentation tanks and facilitates their successful harvest.

Most fungi currently used in fermentation processes grow as pellets and secrete their products into a surrounding fluid. However, some fungi form as long strands or clumps in a filamentous type of growth. It is these fungi for which the biological reactor was designed.

While our hypothetical microbiologist might grow small batches of filamentous-type fungi in laboratory flasks, this is insufficient for industrial

production, says chemical engineer Dennis J. O'Brien, who is at ARS' Eastern Regional Research Center in Philadelphia, Pennsylvania.

O'Brien and co-inventor Wolfgang Heiland, a supervisory mechanical engineer also at the center, have designed a new biological reactor that encourages filamentous-fungal growth.

Currently, industrial fungal fermentations are done by placing the microorganism in a sealed tank filled with liquid containing essential nutrients. The tank is supplied with air, and at the end of fermentation, the mixture is

pumped to a machine that separates the cells from the liquid.

But if filamentous-type fungi are grown under these conditions, the mixture thickens as they grow, reaching a point where it becomes as thick as oatmeal, O'Brien says. The results are poor fungal growth due to lack of oxygen and a mixture that is difficult to further process.

O'Brien says the biomass yield of fungi that form in pellets in current industrial fermentations is about 15 grams per liter, while that of filamentous-type fungi is only about one-third of that.

"If you are basing a process on filamentous fungi, this new reactor offers an economical choice for production," O'Brien says.

A key to the invention is eliminating the need to ferment microorganisms in submerged tanks. Rather, a horizontal cylinder inside the reactor is submerged to half its depth in a nutrient solution.

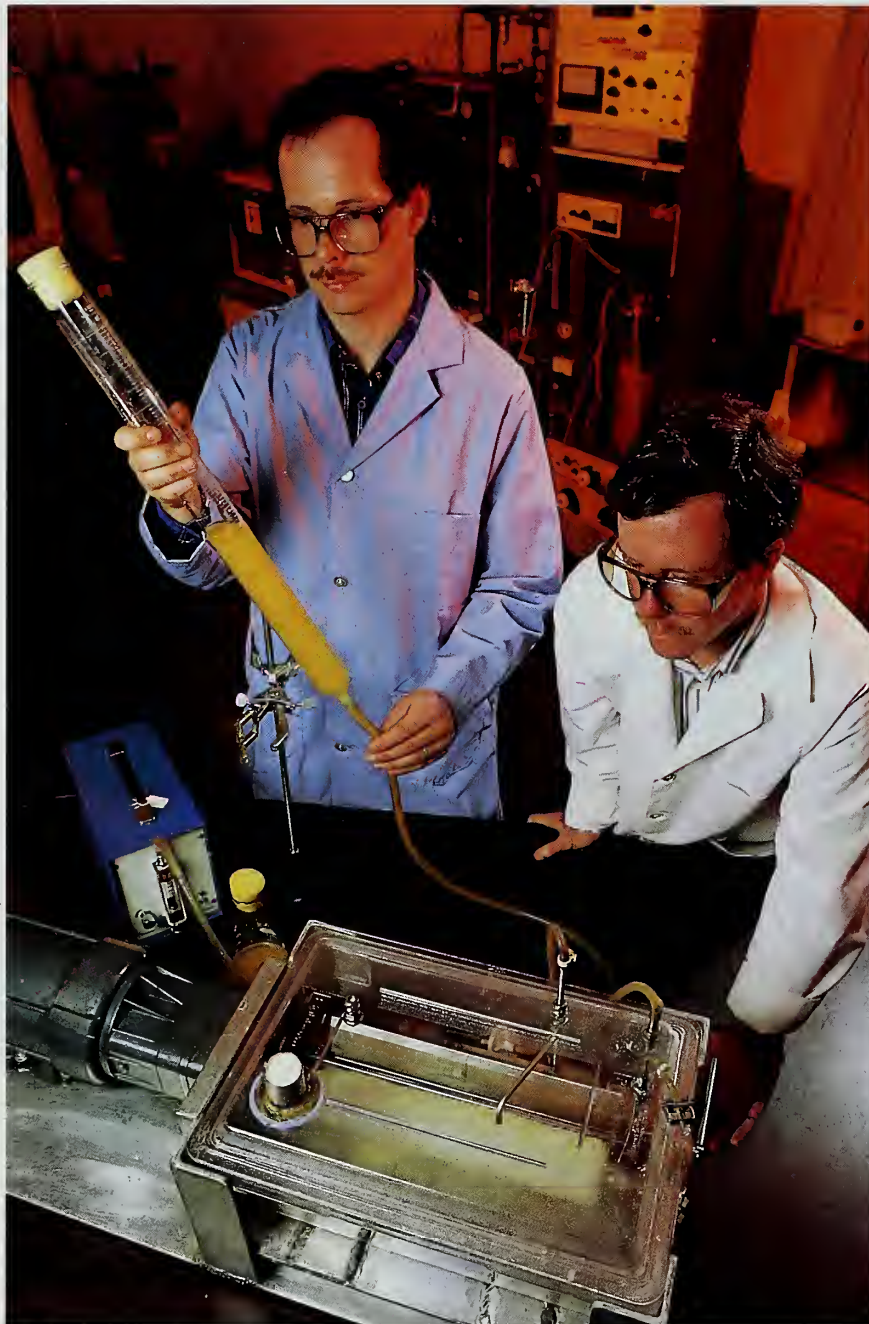
Sterile air is pumped into the reactor through a tube. A shaft continuously rotates the

cylinder, so the fungus gets an equal distribution of nutrients and oxygen, he says.

"It's a better way to grow the organism," O'Brien says. "In submerged fermentation, air is pumped into the solution, but oxygen is not very soluble in water."

Twelve to 19 hours after a fungus is added to nutrient fluid in the reactor, the fungus begins attaching to the rotating cylinder. It continues to grow until it eventually covers the entire cylinder.

SCOTT BAUER



Chemical engineers Dennis O'Brien (right) and Gerard Senske introduce a fungal inoculum into their prototype biological reactor for filamentous-type fungi. (K4707-14)



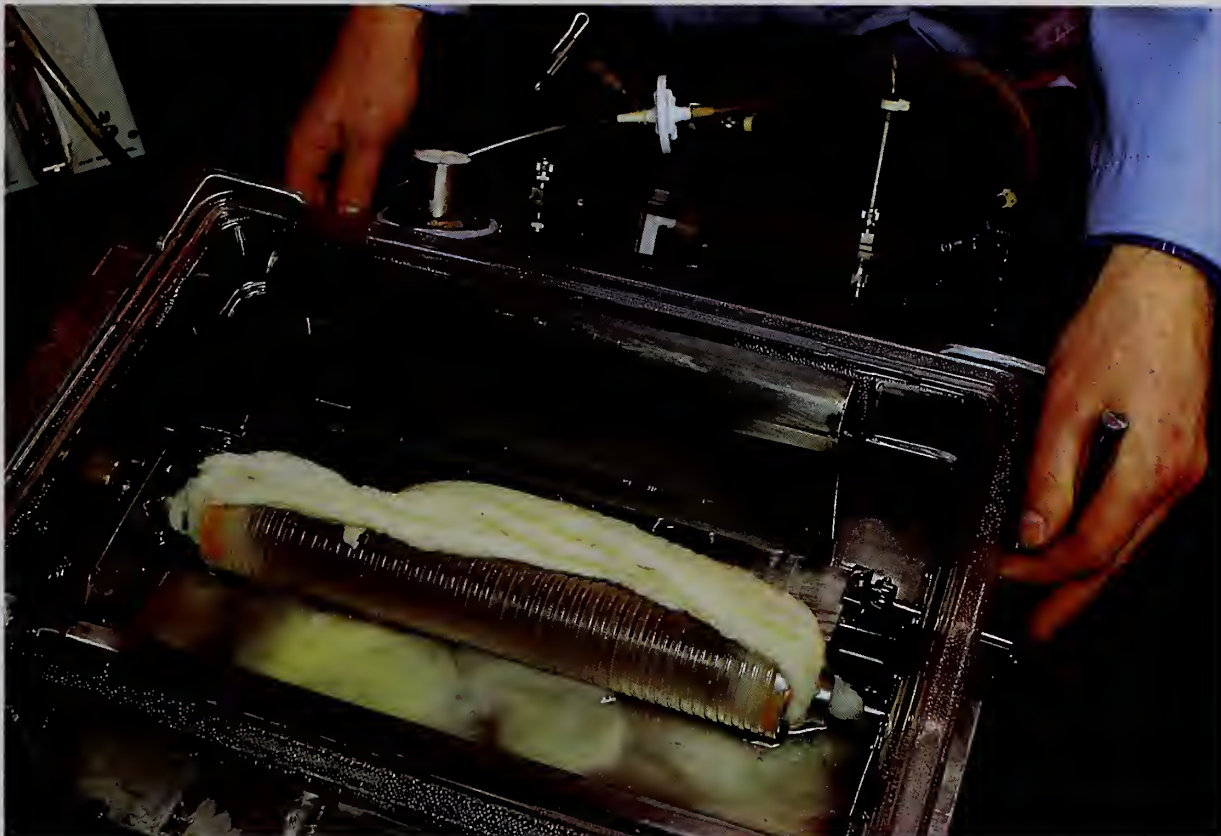
"The fungus grows outward and looks similar to fibers on a paint roller," O'Brien says. "It has the look and feel of raw chicken skin."

The attached material is periodically shaved off the cylinder and caught in a small pan. Once harvested, the fungal growth can be retrieved for extraction of desired compounds.

"Now it's possible to have continuous fungal growth and harvesting," says O'Brien. "This invention gives industry a choice that could improve the economics of fermentation and encourage the development of new microorganisms and products."—By **Bruce Kinzel, ARS.**

*Dennis J. O'Brien and Wolfgang K. Heiland are in the USDA-ARS Engineering Science Research Unit, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118. Phone (215) 233-6601. ♦*

SCOTT BAUER



A steel blade scrapes accumulated fungal growth from the reactor's rotating cylinder core. (K4708-20)

## Fish Oil Product Possible From Fungi

Fungi that can cause disease in the seeds, saplings, and roots of many fruit trees and other crops are being made to serve a useful purpose.

Agricultural Research Service scientists have found that four species of filamentous-type fungi from the genus *Pythium* produce a compound that nutritional research suggests could reduce the risk of heart disease and atherosclerosis.

The compound, known as eicosapentaenoic acid (EPA), is currently obtained from fish oil, says Dennis J. O'Brien, a chemical engineer at ARS' Eastern Regional Research Center in Philadelphia, Pennsylvania.

"Fish oils can have undesirable tastes if not properly processed and

stored," O'Brien says. This adds to their production costs.

So, rather than fishing the oceans for EPA, O'Brien and co-inventors George A. Somkuti, Eric W. Wessinger, and Edward E. Stinson, all at the Philadelphia center, figured out a way to produce EPA without the fish. The four inventors have filed a patent application with the U.S. Patent and Trademark Office on their method for using fungi to efficiently produce EPA as an alternative to fish oil.

The Food and Drug Administration has not yet approved the sale of the compound, but it is being examined in clinical studies sponsored by the National Institutes of Health, says Jacqueline Dupont, ARS' national program leader for human nutrition.

Dupont says that if EPA is shown by these trials to be beneficial, there will be a demand for more than one source of EPA. The compound is currently sold in some countries in Europe.

The process developed at the Philadelphia center involves fermenting the filamentous-type fungi in a biological reactor, harvesting, and extracting EPA-containing oil, says O'Brien. He says the most efficient EPA producer appears to be the species *P. irregulare*.—By **Bruce Kinzel, ARS.**

*Dennis J. O'Brien, George A. Somkuti, Eric W. Wessinger, and Edgar E. Stinson are located at the USDA-ARS Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118. Phone (215) 233-6601.*



# Blowing in the Wind

It comes with the wind.

It happens maybe 10 inches or so above the ground.

And it's one big reason several thousand tons of soil from fallow fields might suddenly blow across the land like grit from a giant sandblaster out to level anything in its way.

Donald Fryrear calls it the TSS Factor. He discovered it and he named it. The letters stand for transition from saltation to suspension.

"It's the point when windblown particles of soil are either blown back to earth or kept aloft," he says. "It's the point in time and space when dust storms really begin."

Soil particles in saltation, explains Fryrear, are too heavy to be carried by the wind. So they return to earth—at a greater speed than when they first took off and with a pulverizing force that kicks finer, lighter particles into the air.

"The whole cycle repeats, building on itself over and over again," Fryrear says. "Eventually, you have a full-fledged dust storm lasting anywhere from minutes to hours—sometimes even for days—and covering maybe hundreds of square miles. But it all starts

when the first bits of soil are swept into the air for a few feet and then get blown back into the ground."


Understanding this initial dynamic in dust storm development, Fryrear says, is critical to predicting the storms and their impact on crops, air quality, and even highway safety.

The TSS factor shows, in particular, how some fallow croplands might contribute significantly to dust storms—even when their surface soil conditions would seem to dictate otherwise. Recent rains, for example, may have caused soil particles to congeal into marble-

sized clumps too big and heavy to allow much in the way of air travel. One might conclude that such congealed particles were less vulnerable to the wind.

But not so. "The small clumps can be the biggest culprits of all," Fryrear says. "They become the instigators, the ground-breaking sledgehammers that make the whole thing happen."

Fryrear, a supervisory agricultural engineer at the ARS Conservation and Production Systems Research Unit in Big Spring, Texas, developed the TSS factor while studying windblown



Ominous and sudden, a roiling dust storm churns up dry, unprotected soil as it bears down on a small Plains town in the midst of the Dust Bowl days. Photo courtesy of the Library of Congress.



soil characteristics at various heights above the ground.

"These were field studies," he points out. "They weren't done in a wind tunnel. I doubt that a wind tunnel approach would have worked. We needed a lot of space, real space and real wind and real soil, to make our data count for something."

Fryrear got what he needed. In addition to outdoor test plots at Big Spring and several fields on nearby farms, his lab became the whole Great Plains





and then some. It included farms and test plots in Oklahoma, Colorado, Kansas, Nebraska, Minnesota, Indiana, Missouri, Florida, New York, Delaware, Arizona, and California—with a little bit of Canada thrown in for good measure.

“In all my years with ARS, and that now comes to nearly 35,” says Fryrear, “I don’t think we’ve ever had another research project on windblown soils involve so many locations spread over so vast an area.”

At each location, Fryrear installed 75 soil particle collectors in a large, circular pattern covering six acres. Such a configuration accommodated winds from any direction, while providing ample space for dust storms to start. The collectors were designed by Fryrear to trap windblown soil particles at different heights up to 40 inches above the ground.

“In some cases, we went up to 20 feet,” he says, “but the first 3 feet or so

were usually enough to go by. The data in that range told virtually the whole story.”

Indeed they did...and a startling story it was.

As expected, windblown soil particles decreased in size the higher the point of collection. But the rate and extent of change was unexpected: Soil particles sampled at the 40-inch level were a thousand times smaller than those rolling along the surface. Furthermore, nearly all of that decrease occurred within the first 10 inches above ground.

“It’s taken 7 years of data to show how often this occurs,” says Fryrear, “and to show how consistent it is with our theory of the TSS factor. Right now, we’re pretty confident about placing the line between suspension and saltation at 10 inches for most types of soil. Our data definitely support this.”

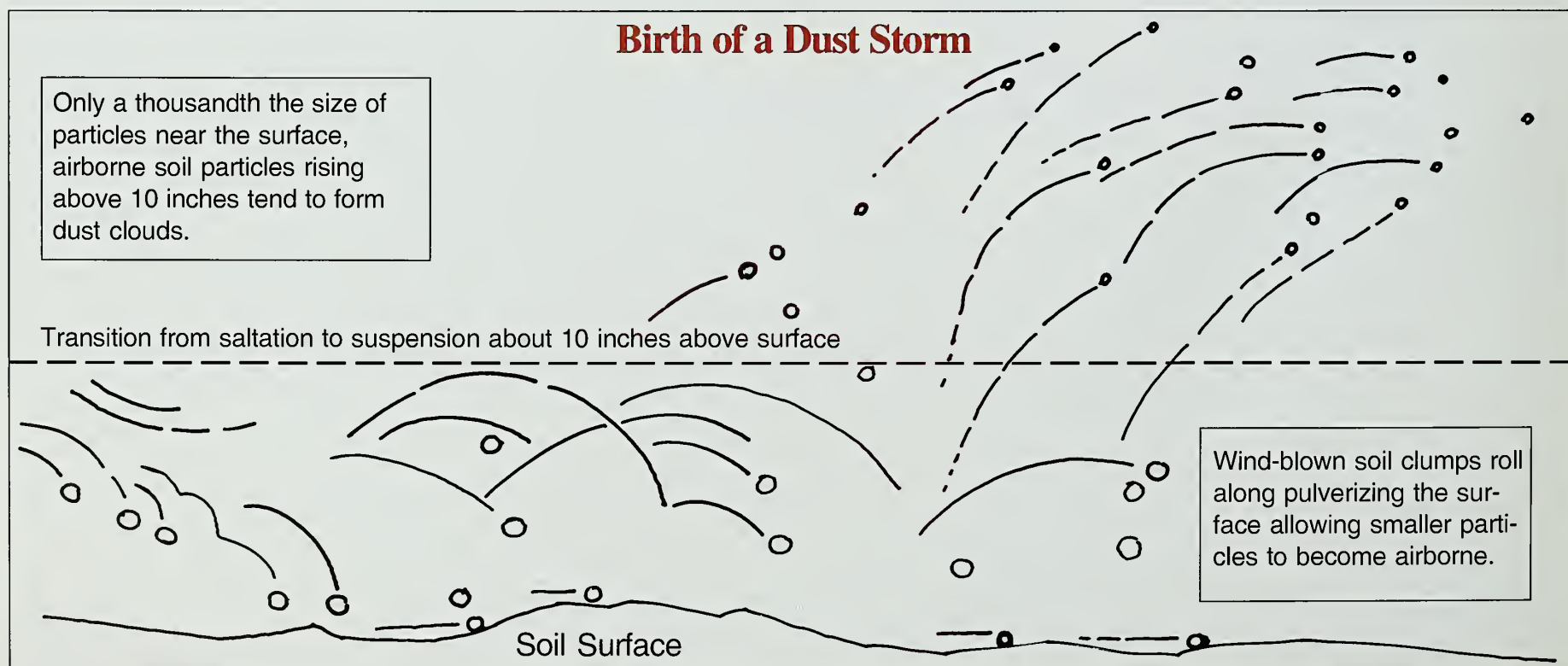
**The TSS Factor is the transition from saltation to suspension... the point in time and space when dust storms really begin.**

If so, monitoring the vertical distribution of windblown soil particles might well become a standard and useful operating procedure in agricultural and semiarid regions where dust storms are most likely to arise.

A significant change in proportions above and below the 10-inch mark at a given location could provide advance warning of an impending dust storm, according to Fryrear. A sudden increase below the mark, for example, would show that larger particles are on the move. And they might be kicking up smaller particles shortly.

“If we had been tracking soil surface conditions in California last year,” says Fryrear, “we might have anticipated the blinding dust storm that hit Interstate 5 suddenly. We might have been prepared. Maybe the highway could have been shut down for a time. Maybe we could have prevented the terrible pileup and wreckage and loss of life that the storm caused.”—By **Stephen Carl Miller, ARS.**

*Donald W. Fryrear is in the USDA-ARS Conservation and Production Research Unit, P.O. Box 909, Big Spring, TX 79721. Phone (915) 263-0293. ♦*





## Protecting Orchards Against Nematodes

Microscopic worms called nematodes are weakening and killing almond, peach, and plum trees in some California orchards. The threadlike worms feed on tree roots by inserting stylets, that resemble hollow needles, to extract juices.

Growers have few remaining weapons for fighting this underground menace. Their use of soil fumigants to kill nematodes has been increasingly restricted because these chemicals may seep into rivers and streams, says ARS scientist Craig A. Ledbetter at Fresno, California.

He'd like to protect tomorrow's orchards with rootstocks that are naturally resistant to the aggressive worms. Rootstocks are the lower, rooted portion of trees, to which the upper, fruit- or nut-bearing budwood, or scionwood, is grafted.

The ideal rootstock, Ledbetter says, would offer resistance to root knot, root lesion, and ring nematodes. If unchecked, these three species pose the biggest economic threat to California orchards of almonds, peaches, plums, nectarines, and apricots.

California is the nation's leading producer of these crops, valued at about \$1 billion annually.

Working with Michael V. McKenry of the University of California at Riverside, Ledbetter has found nine candidate rootstocks that fend off the root lesion nematode—the species he's targeted first. The rootstocks outperformed some 150 others that he grew from seeds or cuttings supplied by ARS collections in Pullman, Washington, Glenn Dale, Maryland, and Davis, California.

The best of the contenders is Bruce, a descendant of a native American plum. In greenhouse tests, Ledbetter exposed newly rooted cuttings to attack by root lesion nematodes.

After 5 months, Bruce had only one-tenth as many root lesion nematodes as Mariana 2624, a popular plum rootstock, and one-thirtieth as many as Nemaguard, an ARS-developed rootstock.

Because it is resistant to another enemy—the root knot nematode—

Nemaguard is the most widely planted rootstock in California stonefruit and almond orchards.

Bruce or the other promising rootstocks that can thwart root lesion nematodes may prove easy to graft and meet orchardists' other requirements. If so, Ledbetter will breed Bruce with Nemaguard or similar rootstocks, to give Bruce resistance to root knot nematodes.

He's saving the third culprit—ring nematodes—for last. Breeders have yet to find any almond or stonefruit rootstock resistant to that species. That's why a rootstock with triple nematode resistance, Ledbetter says, may be another 10 or more years away.

Three grower organizations—the California Prune Board, Almond Board of California, and California Tree Fruit Agreement—help fund the research.—By **Marcia Wood, ARS.**

*Craig A. Ledbetter is with the USDA-ARS Horticultural Crops Research Laboratory, 2021 S. Peach Ave., Fresno, CA 93727. Phone (209) 453-3060. ♦*

## Genetic Marker Flags Disease Susceptibility

U.S. soybean growers today—unlike farmers of a generation ago—don't find their crops seriously threatened by bacterial pustule disease, thanks to successes in breeding resistant soybean varieties.

Now research on *Xanthomonas campestris*, the microorganism that causes bacterial pustule, is at the forefront of another advance toward improved soybeans. For the first time, scientists have found that the presence of a certain protein in a soybean marks the soybean as susceptible to a specific disease.

Discovery of the marker protein, a form or isozyme of the enzyme malate dehydrogenase, may help plant breeders quicken the pace of adding genetic diversity to soybeans. "We envision screening large numbers of seeds for the marker protein," says ARS plant geneticist Reid G. Palmer, Ames, Iowa. "Then, only seeds with an altered form of the

marker protein would be used to develop progeny for additional agronomic tests."

Helping Palmer discover the marker were Bradley R. Hedges, a recent graduate student at Iowa State University, and former ARS plant pathologist Sung M. Lim, now at the University of Arkansas.

The test they envision would involve sampling a small portion of each seed that's a candidate for starting a new breeding line and chemically analyzing it for the malate dehydrogenase isozyme using electrophoresis. This widely used laboratory technique uses starch gel and an electrical current to separate proteins by their electrical charges and molecular weights.

One of at least two forms of the malate dehydrogenase and variant forms of other enzymes are present in all plants, playing essential roles in cell components.

In their studies, the scientists systematically crossed either a soybean line resistant to bacterial pustule, or a susceptible line, with lines that were bred to produce variant forms of nine different isozymes.

Then they chemically analyzed seeds of offspring of the crosses. Statistical analyses showed an association or linkage only between bacterial pustule susceptibility and the malate dehydrogenase isozyme.

"Discovery of other genetic markers for susceptibility to other soybean diseases may be in the wings," says Palmer.

At Ames, ARS geneticist Randy C. Shoemaker is using a technique called RFLP (restriction fragment length polymorphism) analysis to identify linkage markers on specific chromosomes for susceptibility to various races of phytophthora root rot.

Genetic puzzles are beginning to come together, Palmer says. However, few markers so far have been linked to traits of specific economic importance.—By **Ben Hardin, ARS.**

*Reid G. Palmer is in the USDA-ARS Field Crops Research Unit, Agronomy Bldg., Iowa State University, Ames, IA 50011. Phone (515) 294-7378. ♦*



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